

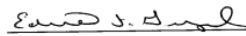
REMARKS

In response to the Notification of Defective Response dated September 25, 3002 requiring a SEQUENCE LISTING in compliance with the requirements of 37 CFR §1.821-1.825, Applicants submit herewith a disk containing the SEQUENCE LISTING in computer readable form (CRF) and a copy of the SEQUENCE LISTING in written form. The transmission cover sheet includes a signed statement of equivalence of the CRF on disk and the attached SEQUENCE LISTING.

The sequences disclosed throughout the specification as filed have been accorded SEQ. ID. NOS. as required in compliance with 37 CFR §1.821. No new matter have been introduced by these amendments.

Entry hereof and examination of the application on the merits are respectfully requested.

Respectfully submitted,


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VERSION OF AMENDMENT WITH MARKINGS TO SHOW CHANGES MADE

For the Examiner's convenience, please note that the additions made are shown in **bold**.

IN THE SPECIFICATION

Please amend the third paragraph beginning on page 6, line 17, as follows:

Figure 3 shows the nucleotide sequence of clone EL14 (**SEQ. ID. NO: 19**) and clone IT2 (**SEQ. ID. NO: 21**). The nucleotide sequence of both clones is aligned with the nucleotide sequence of the germline sequences DP-10 (**SEQ. ID. NO: 20**) (for EL14) and DP-14 (**SEQ. ID. NO: 22**) (for IT2). The different regions of the variable part of the heavy chain are indicated in the following order: framework 1, CDR1, framework 2, CDR2, framework 3, CDR3 and framework 4. Homology of clones EL14 and IT2 with the germline sequences DP-10 and DP-14 is indicated by horizontal bars (-). Differences are indicated by the nucleotides that occur in the germline sequences DP-10 and DP-14. Note that both CDR3 and framework 4 are not derived from the germline sequences DP-10 and DP-14. Consequently, no homology is given for this part of the nucleotide sequence.

Please amend the fourth paragraph beginning on page 6, line 28, as follows:

Figure 4A gives the amino acid sequence (**VHEL-14 (SEQ. ID. NO: 23)** and **VHIT-2 (SEQ. ID. NO: 25)**) derived of the nucleotide sequence of clone EL14 (**SEQ. ID. NO: 19**) and IT2 (**SEQ. ID. NO: 21**). Deviations in the amino acid sequence of the germline segments DP-10 (**SEQ. ID. NO: 24**) and DP-14 (**SEQ. ID. NO: 26**) are indicated in the lower lines. Framework is abbreviated as "FR".

Please amend the last paragraph beginning on page 6, line 32, as follows:

Figure 4B compares the amino acid sequence of three related clones that are derived from the germline segment DP-14 (**SEQ. ID. NO: 26**). The amino acid sequences of clone IT2 (**SEQ. ID. NO: 25**), clone EL5 (**SEQ. ID. NO: 27**) and clone EL25 (**SEQ. ID. NO: 28**) are compared to that of the germline segment DP-14 (**SEQ. ID. NO: 26**). Deviations in

amino acid sequence are indicated for each clone. Note that some amino acid substitutions are shared by the three different clones.

Please amend the first paragraph beginning on page 7, line 1, as follows:

Figure 4C compares the amino acid sequences of the third variable loop (CDR3) of the heavy chain of clone EL14 (SEQ. ID. NO: 29) and IT2 (SEQ. ID. NO: 30).

Homologous amino acid residues are indicated by vertical lines. Dots denote amino acids related in charge or hydrophobicity.

Please amend the third paragraph beginning on page 8, line 21, as follows:

Figure 9A shows the deduced amino acid sequence of recombinant antibody fragments specific for the A3-C1 domain. The amino acid sequence of germline variable heavy chain gene segments DP-15 (SEQ. ID. NO: 31), DP31 (SEQ. ID. NO: 33), DP49 (SEQ. ID. NO: 35) and DP77 (SEQ. ID. NO: 37) is given. Deviations in amino acid sequence from these germline gene segments are indicated for clone B38 (SEQ. ID. NO: 32), B18 (SEQ. ID. NO: 34), B35 (SEQ. ID. NO: 36) and B04 (SEQ. ID. NO: 38). Also the amino acid of the CDR3 and FR4 of the A3-C1 specific recombinant antibodies encoded by clone B38, B18, B35 and B04 is given.

Please delete the fourth paragraph beginning on page 8, line 28, and replace with the following new paragraphs:

Figure 9B gives the nucleotide (SEQ. ID. NO: 39), complementary strand (SEQ. ID. NO: 41), and amino acid (SEQ. ID. NO: 40) sequence of the variable heavy chain domain of clone B38.

Figure 9C gives the nucleotide (SEQ. ID. NO: 42), complementary strand (SEQ. ID. NO: 44), and amino acid (SEQ. ID. NO: 43) sequence of the variable heavy chain domain of clone B18.

Figure 9D gives the nucleotide (SEQ. ID. NO: 45), complementary strand (SEQ. ID. NO: 47), and amino acid (SEQ. ID. NO: 46) sequence of the variable heavy chain domain of clone B35.

Figure 9E gives the nucleotide (SEQ. ID. NO: 48), complementary strand (SEQ. ID. NO: 50), and amino acid (SEQ. ID. NO: 49) sequence of the variable heavy chain domain of clone B04.

Please amend the second paragraph beginning on page 9, line 12, as follows:

Figure 11A shows the deduced amino acid sequence (**SEQ. ID. NOS: 51 and 53**) of recombinant antibody fragments specific for the factor VIII heavy chain. The amino acid sequence of germ line variable heavy chain gene segments DP10 (**SEQ. ID. NO: 24**) and DP47 (**SEQ. ID. NO: 52**) is given. Deviations in amino acid sequence from these germline gene segments are indicated for two clones that encode recombinant antibodies that bind to the factor VIII heavy chain. Also the amino acid of the CDR3 and FR4 of the factor VIII heavy chain specific recombinant antibodies encoded by the two clones is given.

Please amend the third paragraph beginning on page 9, line 20, as follows:

Figures 11B and C give the nucleotide (**SEQ. ID. NOS: 54 and 57**), complementary strand (**SEQ. ID. NOS: 56 and 59**) and amino acid (**SEQ. ID. NOS: 55 and 58**) sequence of the variable heavy chain domain of two clones that encode recombinant antibodies that bind specifically to the factor VIII heavy chain.

Please amend the first paragraph beginning on page 18, line 21 and ending on page 19, line 14, under the heading "EXAMPLE 2", as follows:

Peripheral blood lymphocytes were isolated from a blood sample of a patient with acquired haemophilia. The titre of the inhibitor was 1250 BU/ml. RNA was isolated from the lymphocytes using RNAzol (WAK Chemie, Germany) according to the instructions of the manufacturer. RNA was transcribed into cDNA employing random hexamer primers (Gibco, Breda, The Netherlands). Since, most of the anti-factor VIII antibodies described in Example 1 were of subclass IgG4, DNA fragments corresponding to the heavy chain of immunoglobulins of subclass IgG4 were amplified using the following set of oligonucleotide primers:

conIgG1-4	5'	CTTGTCCACCTTGGTGTGCTGGG	3' (SEQ. ID. NO:1)
huIgG4	5'	ACGTTGCAGGTGTAGGTCTTC	3' (SEQ. ID. NO: 2)

huVH1aback 5'	CAGGTGCAGCTGGTGCAGTCTGG	3' (SEQ. ID. NO: 3)
huVH2aback 5'	CAGGTCAACTTAAGGGAGTCTGG	3' (SEQ. ID. NO: 4)
huVH3aback 5'	GAGGTGCAGCTGGTGGAGTCTGG	3' (SEQ. ID. NO: 5)
huVH4aback 5'	GAGGTGCAGCTGTTGCAGTCGGG	3' (SEQ. ID. NO: 6)
huVH5aback 5'	GAGGTACAGCTGCAGCAGTCTGC	3' (SEQ. ID. NO: 7)
huVH6aback 5'	CAGGTACAGCTGCAGCAGTCAGG	3' (SEQ. ID. NO: 8)
huJH1-2forSal 5'	GAGTCATTCTCGT <u>GTCGAC</u> ACGGTGACCAGGGTGCC	3'
(SEQ. ID. NO: 9)		
huJH3forSal 5'	GAGTCATTCTCGT <u>GTCGAC</u> ACGGTGACCATTGTCCC	3'
(SEQ. ID. NO: 10)		
huJH4-5forSal 5'	GAGTCATTCTCGT <u>GTCGAC</u> ACGGTGACCAGGGTTCC	3'
(SEQ. ID. NO: 11)		
huJH6forSal 5'	GAGTCATTCTCGT <u>GTCGAC</u> ACGGTGACCGTGCGGTCCC	3'
(SEQ. ID. NO: 12)		
huVH1backNeo 5'	AATCCATGGCCCAGGTGCAGCTGGTCCA	3' (SEQ. ID. NO: 13)
huVH2backNeo 5'	AATCCATGGCCCAGGTCAACTTAAGGGA	3' (SEQ. ID. NO: 14)
huVH3backNeo 5'	AATCCATGGCCGAGGTGCAGCTGGTGG	3' (SEQ. ID. NO: 15)
huVH4backNeo 5'	AATCCATGGCCGAGGTGCAGCTGTTGCA	3' (SEQ. ID. NO: 16)
huVH5backNeo 5'	AATCCATGGCCGAGGTACAGCTGCAGCA	3' (SEQ. ID. NO: 17)
huVH6backNeo 5'	AATCCATGGCCCAGGTACAGCTGCAGCA	3' (SEQ. ID. NO: 18)

Please amend the first paragraph beginning on page 19, line 16 and ending on page 20, line 10 as follows:

Oligonucleotide primers huVH_a(1-6)back and huJH(1-6)forSal (SEQ. ID. NOS: 9-12) have been described previously (Marks et al. 1991. J. Mol. Biol. 222: 581-597).

Oligonucleotide primers huVH(1-6)backNeo (SEQ. ID. NOS: 13-18) have been adapted from oligonucleotide primers described in the same paper. The first series of amplification involved primers huVH(1-6)back in conjunction with primer conIgG1-4 (SEQ. ID. NO: 1). Six different DNA fragments of about 700 bp, each corresponding to an individual VH-gene family were obtained. The six different fragments were isolated and re-amplified with primers huVH(1-6)back and primer hulgG4 (SEQ. ID. NO: 2). Six products of

approximately 660 bp were obtained. The 6 different 660 bp fragments which represented the IgG4 repertoire of the patient were re-amplified with primers huVH(1-6)backNco (**SEQ. ID. NOS: 13-18**) and huJH(1-6)forSal (**SEQ. ID. NOS: 9-12**) in order to prepare these fragments for cloning. The resulting 24 fragments were pooled according to VH-gene family and the six different fragments were digested with NcoI and SalI. The digested fragments were purified and dissolved in TE (10 mM Tris-HCl pH=8.0; 0.1 mM EDTA). The vector pHEN-1-VLrep has been described previously (Griffin, H.M. and Ouwehand, W.H. 1995. Blood 86, 4430-4436; Schier et al. 1996. J. Mol. Biol. 255: 28-43) and contains a light chain repertoire derived of two non-immunized donors. Insertion of a heavy chain repertoire in this vector has been shown to result in the production of antibody fragments that consist of the variable domains of both heavy and light chain. These antibody fragments have been termed single chain Fv (scFv) fragments (Hoogenboom, H.R. et al. 1991. Nucleic Acid Res. 19: 4133-4137). The vector pHEN-1-VLrep (kindly provided by Dr. W.H. Ouwehand, Department of Transfusion Medicine, University of Cambridge, UK) was digested with XhoI and NcoI and the six fragments corresponding to the IgG4-specific heavy chain repertoire of the patient with acquired haemophilia were inserted. The ligation mixtures were transformed to *E. coli* TG1 and a library of 1.500.000-2.500.000 independent clones was obtained. Colonies were scraped and resuspended in 2TY supplemented with 15% glycerol, 100 µg/ml ampicillin and 1% glucose. Similar to the methods outlined above libraries that represent the immunoglobulin repertoire of other patients may be assembled.

Please amend the second paragraph beginning on page 21, line 27 and ending on page 23, line 24 as follows:

The second round of panning was initiated by inoculating 50 µl of glycerol stock obtained after the first selection in 10 ml 2TY supplemented with ampicillin (100 µg/ml) and 1% glucose till a final OD600 of 0.3. Cells were grown till an OD600 of 0.5, diluted 1 to 10 in 2TY supplemented with ampicillin (100 µg/ml), kanamycin (25 µg/ml) and 1% glucose and subsequently infected with a 20 fold excess of VCSM13. Cells were grown overnight at 30°C. Supernatant containing the phage was harvested as described above and again screened for binding to immobilized factor VIII light chain. After four rounds of panning, 30 clones selected by ELISA and 30 clones that were obtained after panning with factor VIII

light chain immobilized to immunotubes were grown and analyzed for binding to the factor VIII light chain. Colonies were picked and grown overnight in 2 ml 2TY supplemented with 100 μ g/ml ampicillin and 1% glucose. The next day the cultures were diluted 200 times and grown till an OD600 of approximately 0.5. Cells were subsequently infected with VCSM13 for 45 minutes at 37°C (no shaking) and 45 minutes at 37°C (shaken at 200 rpm). Infected cultures were diluted 1 to 10 in 2TY supplemented with 100 μ g/ml ampicillin, 0.1% glucose and 25 μ g/ml kanamycin and cells were grown overnight at 30°C. Supernatant containing phage was collected after centrifugation and tested for binding to the factor VIII light chain as described below. The non-inhibiting murine monoclonal antibody CLB-CAG 12 directed against an epitope in the A3-C1 domain of factor VIII was immobilized on microtiter wells at a concentration of 5 μ g/ml in 50 mM NaHCO₃ (pH 9.5). Wells were blocked for 1 hour in TBS supplemented with 3% HSA. Wells were incubated with factor VIII light chain at a concentration of 1 μ g/ml in 50 mM Tris HCl (pH 7.4), 1 M NaCl, 2% HSA for 2 hours at 37°C. Fifty μ l of phage solution and an equal volume of TBS supplemented with 1% Tween-20 and 6% HSA were added to wells containing factor VIII light chain. To monitor specific binding of the phages, wells that did not contain factor VIII light chain were incubated simultaneously with the same phage solution. Phage was incubated at room temperature for 2 hours and were shaken at regular intervals. Subsequently, wells were extensively washed 5 times with TBS supplemented with 0.1% Tween-20 and washed 5 times with TBS. The presence of bound phage was monitored by incubating with a peroxidase labelled polyclonal antibody directed against M13 (Pharmacia-LKB, Woerden, The Netherlands) in a dilution of 1 to 4000 in TBS supplemented with 1% HSA and 0.1% Tween-20. Plates were washed 5 times with TBS supplemented with 0.1% Tween-20 and 5 times with TBS. Binding of peroxidase-labelled anti-M13 antibody was quantified by incubation with 3-3'-5-5' tetramethylbenzidine (TMB). Substrate conversion was arrested by the addition of 100 μ l of 2N H₂SO₄. Part of the results of this analysis are given in Figure 1. An example of 12 clones that show specific binding to the factor VIII light chain is given. Clearly, phage encoded by clone 1 to 12 display binding to the factor VIII light chain (black bars). Some background binding is visible which is not dependent on the presence of the factor VIII light chain (grey bars). The bars labelled with c represent two clones that express antibody fragments that do

not bind specifically to the factor VIII light chain. These clones have been derived from the initial library and have not been selected on the factor VIII light chain. To ensure that during subsequent rounds of panning an increase in the amount of factor VIII specific recombinant antibody fragments was obtained, we screened 12 clones obtained after the first round of panning for binding to the factor VIII light chain (Figure 2). Only 3 out of 12 clones bind specifically to the factor VIII light chain. In 9 out of 12 clones binding of phage is not dependent on the presence of the factor VIII light chain. These results clearly indicate that during panning the amount of phages that express factor VIII-specific antibodies can be selectively enriched. In summary, we have outlined a specific protocol for the selection of factor VIII specific antibodies that correspond to the spectrum of anti-factor VIII antibodies present in the patient with acquired haemophilia. In the first two examples our analysis is limited to material derived of one single patient and only antibodies directed against the factor VIII light chain have been analyzed. Using the methods outlined in these two examples the repertoire of anti-factor VIII antibodies of other patients with an inhibitor can easily be obtained. Furthermore, anti-factor VIII antibodies directed against epitopes located outside the factor VIII light chain may be obtained by adapting the screening methods used in Example 1 and 2. Antibodies directed against the heavy chain can be selected by immobilizing factor VIII heavy chain employing monoclonal antibody CLB-CAg 9. In these two examples we have focused on the IgG4-repertoire of the patient. Similarly, other subclasses may be investigated using the appropriate primers. For example, subclass IgG1-4 can be detected by simply using primer conIgG1-4 (SEQ. ID. NO: 1) described in Example 1. Similarly, other primers specific for IgA, IgM, IgE and IgD may be utilized to assemble antibody-repertoires that include factor VIII-specific antibodies.

Please amend the first paragraph beginning on page 23, line 28 and ending on page 25, line 2 as follows:

In the previous examples methods to obtain recombinant antibodies with factor VIII specificity has been outlined. To obtain information on the properties of these antibodies we selected 30 clones that have been selected by immobilized factor VIII in immunotubes. Also 30 clones which were selected employing factor VIII light chain with monoclonal antibody CLB-CAg 12 were analyzed. Clones were grown as described in Example 2 and plasmid

DNA was isolated. The nucleotide sequence of the variable part of the heavy chain (VH domain) of 55 clones was determined using fluorescently labelled M13 reverse primer on an ABI-Prism 377 DNA sequencer. The sequences obtained were aligned with heavy chain sequences in the database "V BASE" of the MRC Centre of Protein Engineering (Cambridge, UK). The 55 clones analyzed were encoded by two different VH-gene segments DP-10 (**SEQ. ID. NO: 20**) and DP-14 (**SEQ. ID. NO: 22**) (Cook and Tomlinson, Immunology Today 16: 237-242). The 41 clones that were encoded by the germline sequence DP14 (**SEQ. ID. NO: 22**) consisted of three groups of recombinant antibodies that differed mainly in the nucleotide sequences of the constant regions of the VH gene. Thirty-three clones which were represented by clone IT2, 5 clones were represented by EL25 and 3 clones were represented by clone EL5 (Table I). Two clones that were encoded by DP10 (**SEQ. ID. NO: 20**) (EL14) (**SEQ. ID. NO: 19**) and DP14 (**SEQ. ID. NO: 22**) and (IT2) (**SEQ. ID. NO: 21**) were selected for further analysis. The nucleotide and primary amino acid sequence of these clones is listed in Figure 3 and 4. The characteristics of the two sequences are given in Table I. Part of clone EL14 is most likely derived of the D-segment D6-13 and J-segment JH-3b. Somatic hypermutation has occurred during the immune response as evidenced by the large number of nucleotide changes compared to the germline sequences of the VH segments. The variable heavy chain part of clone IT2 (**SEQ. ID. NO: 21**) contains 20 nucleotide substitutions when compared to the germ line segment DP-14 (**SEQ. ID. NO: 22**). These 20 nucleotide substitutions result in a total of 13 amino acid changes (Table I). The variable heavy chain part of clone EL14 (**SEQ. ID. NO: 19**) contains 18 nucleotide substitutions when compared to the germ line segment DP-10 (**SEQ. ID. NO: 20**). These 18 nucleotide substitutions result in 12 amino acid changes (Table I). Clone IT2 has in part been derived from gene segments D3-3 and JH6b. Remarkably, a stretch of G-residues is observed between the germ line sequences DP14 (**SEQ. ID. NO: 22**) and D3-3 for clone IT2 that encodes for a flexible arm of glycine residues. Inspection of the amino acid sequence of clone EL14 (**SEQ. ID. NO: 23**) and IT2 (**SEQ. ID. NO: 25**) reveals several interesting features. Both CDR3 regions contain several glycine residues at their amino-terminal part which is in both cases followed by a tyrosine and a glutamic acid (GG-YE). Furthermore, a proline, alanine and an aspartic acid appear to be conserved in the carboxyl-terminal part of the CRD3 (P---A-D). A common motif can be derived from the amino acid sequences of the

CDR3 regions of clone EL14 (**SEQ. ID. NO: 23**) and IT2 (**SEQ. ID. NO: 25**) which is given in Figure 4B. These features may determine the specificity of these antibodies for the factor VIII light chain. In this example the nucleotide and primary amino acid sequence of two recombinant factor VIII antibodies has been disclosed. With methods similar to the ones described in this example recombinant antibodies that are directed against other regions on the factor VIII molecule may be analyzed. Common features of these antibodies can be identified as outlined in this example and therapeutic and diagnostic agents derived of these common features can be used for diagnosis and treatment of patients with factor VIII inhibitors.

Please amend the first paragraph beginning on page 25, line 6 and ending on page 26, line 12 as follows:

The nucleotide and amino acid sequences outlines in the previous example can be used to specifically detect factor VIII antibodies with C2-specificity in heterogeneous mixtures of antibodies. This can be accomplished by developing reagents, for example, antibodies that specifically recognize the anti-factor VIII antibodies described in this invention. Detection of factor VIII-specific antibodies can also be performed by analysis of the presence of specific nucleotide sequences that encode factor VIII specific antibodies. Methods to obtain nucleotide sequences that encode factor VIII specific antibodies are disclosed in this invention. In this example the detection of nucleotide sequences encoding one of the factor VIII specific antibodies described in the previous example (EL14) (**SEQ. ID. NO: 19**) is disclosed. Lymphocytes of the patient with acquired haemophilia described in the first example were obtained. RNA was isolated and cDNA was prepared. Subsequently, DNA fragments were amplified with oligonucleotide primers huVH(1-6)aback (**SEQ. ID NOS: 3-8**) and conIgG1-4 (**SEQ. ID. NO: 1**) (see Example 2). The six different 700 bp fragments obtained were isolated and used for a second PCR with oligonucleotide primer huVH(1-6)aback (**SEQ. ID. NOS: 3-8**) and huIgG4 (**SEQ. ID. NO: 2**). This resulted in a fragment of 660 bp which was cloned into the vector pGEM-T (Promega, Madison, WI, USA). The presence of nucleotide sequences that corresponded to that of clone EL14 (**SEQ. ID. NO: 19**) was addressed by nucleotide sequencing. One out of sixty

clones analyzed did contain nucleotide sequences that were identical to that obtained for clone EL14 (**SEQ. ID. NO: 19**). This analysis shows that, using the nucleotide sequences disclosed in this invention as a starting point, it is possible to monitor the presence of factor VIII specific antibodies in patient samples. In this example oligonucleotide primers are used which have also been employed for the construction of the IgG4 specific library. Other combinations of oligonucleotide primers that are based on the nucleotide sequences of clone EL14 (**SEQ. ID. NO: 19**) and IT2 (**SEQ. ID. NO: 21**) may be designed which may include but are not limited to oligonucleotide primers that are based upon the CDR3 region of these antibodies. In this example detection of factor VIII specific antibodies is performed using analysis of nucleotide sequences. Alternatively, detection of factor VIII specific sequences may also be performed employing selective hybridization using probes that are based on the nucleotide sequence of the factor VIII specific antibodies disclosed in this invention. Other means of detection of specific nucleotide sequences that are known to an average expert in the art also fall within the scope of this invention. The methods disclosed in this invention allow for the isolation of factor VIII antibodies and determination of their nucleotide and amino acid sequence. In this example we have outlined described methods that detect factor VIII specific antibodies present in the repertoire of a patient with a factor VIII inhibitor. In examples 8 and 9 the nucleotide sequence of antibody fragments that bind to the A2- and A3-C1 domain of factor VIII is given. Methods similar to the ones described in this example can be used to detect nucleotide sequences that encode factor VIII inhibitors with A2-, A3-C1- or with a different epitope-specificity.

Please amend the first paragraph beginning on page 28, line 16 as follows:

Next, different dilutions of scFv-EL14 and scFv-IT2 were tested for binding to immobilized factor VIII light chain as outlined above using CLB-CAg A as the detecting antibody (Figure 6). From this analysis it appeared that scFv-EL14 binds with a higher affinity to the factor VIII light chain than scFv-IT2. These results were complemented by immunoprecipitation experiments for scFv-EL14. Immunoprecipitation experiments employing a metabolically labelled fragment corresponding to the C2-domain was performed essentially as described previously (Fijnvandraat et al. 1998. Blood 91: 2347-2352).

Monoclonal antibody 9E10 was covalently linked to CNBr-activated Sepharose 4B and this

matrix was used to bind scFv-EL14. Specific binding of scFv-EL14 to metabolically labelled C2-domain was detected and this confirms the C2-specificity of this recombinant antibody fragment. In this example methods have been disclosed to characterize recombinant antibodies with specificity for the C2-domain. In examples 8 and 9, we describe the nucleotide and amino acid sequence of recombinant antibody fragments that bind specifically to the A2- (**SEQ. ID. NOS: 54-59**) and A3-C1 (**SEQ. ID. NOS: 39-50**) domain of factor VIII. The methods described in this example can easily be adapted by an average expert skilled in the art, which will allow for characterization of recombinant antibodies directed against the A2 (**SEQ. ID. NOS: 54-59**), A3-C1 (**SEQ. ID. NOS: 39-50**) or another epitope on factor VIII.

Please amend the first paragraph beginning on page 29, line 3 and ending on page 30, line 4 as follows:

In the previous example, we have shown that scFv-EL14 and scFv-IT2 bind to the factor VIII light chain and compete for binding with the murine inhibitory monoclonal antibody CLB-CAg 117. These observations suggest that the epitope of both scFv-EL14 and scFv-IT2 overlaps with that of CLB-CAg 117. It is expected that similar to CLB-CAg 117, scFv-EL14 and scFv-IT2 inhibit the biological activity of factor VIII. Increasing amounts of purified scFv's were tested for inhibition in the Bethesda assay. Surprisingly, addition of up to 170 µg/ml scFv did not result in factor VIII inhibition as measured in the Bethesda assay. In contrast, CLB-CAg 117 readily inhibited factor VIII when measured in the same assay. Apparently, binding of scFv-EL14 and scFv-IT2 to factor VIII does not interfere with the biological activity of factor VIII. This finding prompted us to investigate the capacity of both scFv-EL14 and scFv-IT2 to overcome inhibition by CLB-CAg 117. Monoclonal antibody CLB-CAg 117 was diluted till a final inhibitory activity of 2 BU/ml. This value corresponds with a residual factor VIII activity of 25% in the Bethesda assay. Subsequently, increasing concentrations of scFv-EL14 and scFv-IT2 were added. Surprisingly, both scFv-EL14 and scFv-IT2 could overcome the factor VIII inhibitory activity of CLB-CAg 117 (Figure 7). ScFv-14 (panel A) proved to be more efficient than scFv-IT2 (panel B) in neutralizing the inhibitory activity of CLB-CAg 117. Both scFv-EL14 and scFv-IT2 were unable to neutralize the inhibitory activity of monoclonal antibody CLB-CAg A, directed against amino

acid residues Glu¹⁸¹¹-Lys¹⁸¹⁸ on the factor VIII light chain (Lenting et al. 1996. *J. Biol. Chem.* 271:1935-1940). These results for the first time show that antibody fragments with factor VIII specificity can be used to interfere with the activity of factor VIII inhibitors. Administration of these antibody fragments will be beneficial for the treatment of patients with inhibitory antibodies directed against factor VIII. In this example the biological activity of antibody fragments with C2-specificity is disclosed. In examples 8 and 9, the nucleotide and amino acid sequence of recombinant antibody fragments that bind to the A2 (**SEQ. ID. NOS: 54-59**) and A3-C1 (**SEQ. ID. NOS: 39-50**) domain of factor VIII is disclosed. The methods disclosed in this and the previous example can easily be adapted by an average expert skilled in the art to establish the capacity of recombinant antibody fragments directed against the A2 or A3-C1 domain to neutralize factor VIII inhibitors. Similar to outlined in this example recombinant antibody fragments that bind to other regions can be evaluated for their neutralizing capacity of factor VIII inhibitors. Similarly to what has been described in this example for scFv-EL14 and scFv-IT2, antibody fragments binding to A2, A3-C1 and other domains on factor VIII can be used for treatment of patients with factor VIII inhibitors.

Please amend the second paragraph beginning on page 32, line 21 as follows:

The nucleotide sequence of the variable heavy chain fragments of 26 clones that reacted specifically with recombinant A3-C1 domain was determined essentially as described in Example 4. The sequences obtained were aligned with heavy chain sequences in the database "V BASE" of the MRC Centre of Protein Engineering (Cambridge, UK). The 26 clones analyzed were encoded by four different VH-gene segments DP15 (**SEQ. ID. NO: 31**), DP31 (**SEQ. ID. NO: 33**) and DP49 (**SEQ. ID. NO: 35**) and DP77 (**SEQ. ID. NO: 37**). The amino acid sequence of the variable heavy chain fragments of clones B38 (**SEQ. ID. NO: 32**), B18 (**SEQ. ID. NO: 34**), B35 (**SEQ. ID. NO: 36**) and B04 (**SEQ. ID. NO: 38**) is listed in Figure 9A. The nucleotide sequence of these four clones is presented in Figures 9B-E (**SEQ. ID. NOS: 39, 42, 45, 48**).

Please amend the second paragraph beginning on page 34, line 33 and ending on page 35, line 4 as follows:

The nucleotide sequence of the variable heavy chain fragments of 26 clones that reacted specifically with the factor VIII heavy chain were determined essentially as described in Example 4. The sequences obtained were aligned with heavy chain sequences in the database "V BASE" of the MRC Centre of Protein Engineering (Cambridge, UK). The 26 clones analyzed were encoded by two different VH-gene segments DP10 (**SEQ. ID. NO: 24**) and DP47 (**SEQ. ID. NO: 52**) (Figure 11A). The nucleotide sequence of the variable heavy chain of these clones is listed in Figure 11B (**SEQ. ID. NO: 54**) and C (**SEQ. ID. NO: 57**).

Please delete the table on page 37 and insert the following new table as follows:

Table I: Nucleotide sequences of clones expressing recombinant antibodies with specificity for the factor VIII light chain. Based on the nucleotide sequence 55 of the 60 clones analyzed could be arranged as depicted below. In the first column clones with the same nucleotide sequence are arranged in four groups. The number of clones corresponding to this group is given in brackets. Clone EL5 (**SEQ. ID. NO: 27**), EL25 (**SEQ. ID. NO: 28**) and IT2 (**SEQ. ID. NO: 25**) are related as indicated in Figure 4B. In the second column the heavy chain family to which these clones belong is depicted. All clones analyzed belong to the VH1-family. In the third column the germline segment is depicted. Clone EL5 (**SEQ. ID. NO: 27**), EL25 (**SEQ. ID. NO: 28**) and IT2 (**SEQ. ID. NOS: 21, 25**) belongs to germline segment DP-14 (**SEQ. ID. NOS: 22, 26**) while clone EL14 (**SEQ. ID. NOS: 19, 23**) belong to germline sequence DP-10 (**SEQ. ID. NOS: 20, 24**). In the fourth column the number of mutations in the different clones is depicted. The first number corresponds to the number of nucleotide mutations while the second one corresponds to the number of amino acid changes. The sequences were compared with the nucleotide and amino acid sequences of the germline segments indicated in the Table.

Clone	VH family	Germline segment	Mutations
EL5 (3)	VH1	DP-14	20/12
EL14 (14)	VH1	DP-10	18/12
EL25 (5)	VH1	DP-14	19/11
IT2 (33)	VH1	DP-14	20/13